

EFFECT OF FUNGICIDE SEED TREATMENTS ON *FUSARIUM VIRGULIFORME*  
AND DEVELOPMENT OF SUDDEN DEATH SYNDROME IN SOYBEAN

BY

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THESIS

Submitted in partial fulfillment of the requirements  
for the degree of Master of Science in Crop Sciences  
in the Graduate College of the  
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

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## ABSTRACT

Sudden death syndrome (SDS) is a soilborne disease responsible for causing significant yield reductions across soybean-producing states. Recent research indicates that infection of the soybean radicle early in the season by *Fusarium virguliforme* (Fv), the SDS pathogen, is critical for disease development. This suggests fungicide seed treatments could be effective in limiting early infection possibly resulting in disease control. Field, greenhouse, and laboratory studies were conducted to evaluate eight fungicides in multiple seed treatment combinations for effects on Fv infection and SDS development. Seed treatments were applied to cultivars that were moderately resistant and moderately susceptible to SDS. Field studies were conducted at two locations: in 2008, the Valmeyer, IL location was naturally infested with Fv, and in 2008 and 2009 the Urbana, IL location had a natural Fv infestation and soil was augmented with sterilized grain sorghum colonized by Fv. Similarly, the greenhouse study was inoculated with sterilized, Fv infested grain sorghum to evaluate the same seed treatments on moderately resistant and moderately susceptible cultivars. The rolled-paper towel laboratory assay tested the individual fungicides in the growth chamber using a Fv macroconidial suspension to inoculate treated seed and assess effects on seed germination, plant length, lesion size, and disease severity. Fv DNA concentrations in soybean roots were measured using quantitative polymerase chain reaction (qPCR) in early vegetative stage roots. Soybean roots from the field were collected at three timings for digital scanning and analysis with root analysis software to measure root disease symptoms. Roots were scanned and analyzed at the completion of the greenhouse trial. SDS foliar symptoms were rated several times throughout plant growth and the area under disease progress

curve (AUDPC) was calculated. Harvest data were collected for the field study. In the field, seed treatments had no effect on Fv DNA concentrations in roots. Seed treatments had very little effect on roots analyzed from the field. Most seed treatments did significantly decrease SDS foliar symptoms at the Valmeyer field study compared to the control, but no differences in foliar symptoms were observed for the Urbana field studies. Yield was unaffected by seed treatments. In the greenhouse, Fv DNA concentrations were reduced by a treatment combining mefenoxam + thiophanate-methyl + azoxystrobin + *Bacillus pumilus* + prothioconazole + fludioxonil compared to the non-treated control; however, the reduction in Fv DNA did not improve root growth or decrease SDS foliar symptoms compared to the non-treated control. The Fv DNA concentrations in roots did not significantly correlate to SDS foliar symptoms in the field; however, a significant positive correlation was found in the greenhouse between Fv DNA and SDS foliar symptoms. Several seed treatments decreased lesion length and disease severity compared to the non-treated inoculated control in the rolled-towel laboratory assay, but the biological seed treatment, *B. pumilus*, significantly decreased seed germination and plant length while increasing lesion length and SDS severity compared to the non-treated inoculated control. In conclusion, none of the seed treatments evaluated proved to have consistent effects on Fv or SDS.

## **ACKNOWLEDGMENTS**

I would like to thank Keith Ames, Dianne Pedersen, Dr. Venkat Chapara, Guirong Zhang, and all the past and present members of Dr. Carl Bradley's research group for their technical support in this research, none of which would have been possible without their help. I also would like to thanks those members of Dr. Jason Bond's research group for their support at the Valmeyer, IL location. I especially appreciate the technical support and advice I received from Dr. Glen Hartman and Dr. James Haudenshield concerning molecular laboratory techniques and analysis. I would like to thank the members of my committee, Dr. Darin Eastburn, Dr. Jerald Pataky, and Dr. Terry Niblack, as well as Kevin Armstrong and Dr. Matthew Maughan from the Crop Sciences' statistics group. Above all, I would to express my gratitude to my adviser, Dr. Carl Bradley, for his tireless support and patience. None of this research would have been possible without the financial support of the Illinois Soybean Association.

On a more personal level, I would like to thank Anni, my parents, Darrell and Della Weems, and my siblings, Jason, Heather, Josh, Jed, and Jared. I could not have completed this project without their love and support.

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## INTRODUCTION

Soybean (*Glycine max*) is a major grain crop in the world. In 2010, 258.4 million tonnes of soybean were produced worldwide, accounting for 58% of the world's oilseed production (American Soybean Association, 2011). The majority of the soybeans were produced in the United States, Brazil, Argentina, China, India, Canada, and Paraguay. In 2011, the United States led in soybean production, producing 90.6 million tonnes, and Illinois was the second leading producer of soybeans in the U.S. that year, producing 12.68 million tonnes in 2010 (American Soybean Association, 2011). Soybean yields are frequently limited by disease, including sudden death syndrome (SDS). SDS is an important disease which has recently caused considerable yield loss in soybean in Illinois. Evaluating new management techniques for control of SDS may aid in development of disease control strategies that will allow growers to manage soybean acres more productively and profitably.

**Causal agent(s) of SDS.** In 1971, an unknown disorder of soybean causing interveinal chlorosis and necrosis of leaves and defoliation was observed in Arkansas (Rupe, 1989; Roy et al., 1997; Rupe and Hartman, 1999). The disease was formally named sudden death syndrome (SDS) after it continuously caused defoliation and pod abortion resulting in reduced yields in east central Arkansas (Hirrel, 1983). In 1988, the causal agent of SDS was determined to be *Fusarium solani*, a soilborne, imperfect fungus (Roy et al., 1988). Based on host specialization and plant symptoms, Roy et al. (1997) concluded that *F. solani* strain A caused SDS and designated it *F. solani* f. sp. *glycines*.

Aoki et al. (2003) compared DNA sequence from the nuclear ribosomal intergenic spacer region and the single copy nuclear gene translation elongation factor 1-alpha of

SDS pathogens from North and South America. These comparisons were combined with a detailed comparison of conidial morphology to prove the pathogens causing SDS on the two continents were different. The causal agents of SDS were reclassified as *Fusarium virguliforme* (Fv) in North America and *Fusarium tucumaniae* in South America (Aoki et al., 2003). Aoki et al. (2005) has since isolated the North American SDS fungal species (Fv) in South America, as well as two new species, *F. brasiliense* and *F. cuneirostoma*. Although the four species are morphologically and phylogenetically distinct within clade 2 of the *F. solani* species complex, they all are causal agents of SDS in soybean (Aoki et al., 2005).

**Symptoms and signs of SDS.** SDS is characterized by pronounced foliar symptoms. These symptoms generally develop during reproductive growth stages in scattered patches throughout fields (Rupe and Gbur, 1995). Symptoms begin on upper leaves; these interveinal, irregularly-shaped, scattered, chlorotic spots a few millimeters in diameter produce a mottled appearance (Roy et al., 1997; Rupe and Hartman, 1999). Young leaves may also display marginal cupping (Rupe and Hartman, 1999). Chlorotic spots later expand and become necrotic until only the tissue surrounding the major leaf veins remain green. Severely diseased leaves may drop off, leaving petioles attached to the plant (Roy et al., 1997; Rupe and Gbur, 1995; Rupe and Hartman, 1999). Onset of the disease during early plant reproductive stages may lead to flower and pod abortion beginning with uppermost flowers and pods. Later onset of the disease can result in decreased seed size, complete defoliation, and premature death, leaving the plant susceptible to infection by pathogens that cause late harvest diseases, such as *Phomopsis* and *Diaporthe* species (Roy et al., 1997; Rupe and Hartman, 1999; Luo et al., 2000).

Diseased plants can display symptoms and signs of the fungus on the root, as well. The root vascular tissue becomes discolored, turning gray to reddish-brown. This discoloration can extend from the lateral roots and taproot up several nodes of the stem, while the pith of the roots remains white (Navi and Yang, 2008; Roy et al., 1997; Rupe et al., 1993; Rupe and Hartman, 1999). Severe infection can cause root necrosis and significantly reduce root length, surface area, volume, and mass in infected plants, which can be easily pulled from the ground (Ortiz-Ribbing and Eastburn, 2004). On the surface of severely infected roots and lower stems, the pathogen produces blue-green sporodochia (Roy et al., 1997; Rupe and Hartman, 1999; Scherm et al., 1998).

**SDS disease cycle.** Chlamydospores act as primary SDS inoculum and overwintering structures in soil (Aoki et al., 2003). The population density of chlamydospores in soil is directly correlated to SDS severity (Scherm et al., 1998) because germinating soybean seedlings intercept the inoculum as they grow. Roots are directly infected (Roy et al., 1997), and colonization can take place as early as seedling germination (Gao et al., 2006; Li and Hartman, 2003; Navi and Yang, 2008). Early seedling infection allows the pathogen to colonize the xylem, resulting in foliar symptom expression (Navi and Yang, 2008; Gongora-Canul and Leandro, 2011). Foliar symptoms are the result of fungal phytotoxins translocated through the xylem to leaves (Baker, 1994; Jin et al., 1996; Li et al., 1999; Navi and Yang, 2008). Reproduction takes place on root surfaces, forming macroconidia in sporodochia (Roy et al., 1997). Chlamydospores are produced from these macroconidia in the presence of soil exudates (Melgar et al., 1994). Hyphal growth is limited to the roots and crown tissue (Ortiz-



Ribbing and Eastburn, 2004), where chlamydospores also are produced (Rupe and Hartman, 1999).

Pathogen infection and disease development are favored by cool, wet conditions in locations with previous history of SDS. High soil moisture and low soil temperatures at early vegetative and reproductive stages increase SDS development (Roy et al., 1997; Rupe and Hartman, 1999; Scherm and Yang, 1996; Scherm et al., 1998), and Fv inoculum concentration in the soil positively correlates to disease pressure (Hershman et al., 1990; Wrather et al., 1995). SDS is commonly reported in irrigated, fertile fields with high yield potential (Hirrel, 1983; Melgar et al., 1994; Scherm and Yang, 1996). Soybeans planted continuously or in corn/soybean rotations also are more susceptible due to Fv inoculum build-up (Rupe et al., 1997). SDS incidence and severity also are dependent on the susceptibility of the cultivar (Rupe et al., 1991).

*Heterodera glycines* (soybean cyst nematode; SCN) infection has been shown to increase the severity of SDS. The interaction was first suggested by Hirrel (1987) in the early 1980s when more severe SDS symptoms were observed in fields with SCN. McLean and Lawrence (1993) found that plants co-infected with SCN and Fv developed SDS symptoms sooner and with greater incidence and severity than plants inoculated with Fv alone. Similar conclusions have been reached in other studies as well (Melgar et al., 1994; Xing and Westphal, 2006). Fv also can infect *H. glycines* cysts, which may act as overwintering sites for the pathogen (McLean, 1993; Roy et al., 1997). Conversely, high levels of SDS have been shown to decrease the population of SCN by reducing root mass, limiting SCN infection sites (Gao et al., 2006).

**Distribution of SDS.** SDS has spread across soybean production areas in North and South America since its identification in 1971. In 1994, Argentina, Brazil, and the U.S. were the only countries to report yield losses due to SDS, but by 1998, the estimated annual losses caused by SDS had increased in all three countries (Wrather et al., 2001). SDS also began causing yield losses in Canada and Paraguay in the 1990s (Wrather et al., 2001). Yield reductions in 1998 were estimated to be 1,254,600 tonnes worldwide (Wrather et al., 2001).

In the U.S., SDS is widespread and reduces soybean yield in many states. SDS was first reported in Arkansas in 1971 (Roy et al., 1997), and was initially considered a disease limited to the southern U.S. It has since spread to the north and was identified in Illinois in the mid-1980s (Eathington et al., 1993). Most recently, SDS was identified in Minnesota in 2003 (Kurle et al., 2003), Wisconsin in 2007 (Bernstein, 2007), and Michigan in 2008 (Chilvers and Brown-Rytlewski, 2010). Scherm and Yang (1999) concluded that conditions in the north-central U.S. are favorable for disease development, and that the main area of damage will not be restricted to southern states. Losses from SDS are typically more severe in the northern U.S. due to the cooler, wetter climate and soil conditions (Scherm and Yang, 1999). From 2003 to 2005, SDS reduced yields by 1,721,000 tonnes in northern states and only 173,000 tonnes in southern states (Wrather et al., 2006).

**SDS management.** Several cultural practices are used to reduce the severity of SDS. Rupe et al. (1997) found that crop rotation could reduce SDS by limiting soil populations of *Fv* and *H. glycines*. Rotations to *Sorghum bicolor* and *Triticum aestivum* reduced *Fv* populations most significantly, but most non-soybean crops lowered *H.*

*glycines* populations (Rupe et al., 1997). Surface tillage and deep tillage to breakup claypans can reduce the occurrence of SDS by reducing soil moisture and promoting soil heating (Von Qualen et al., 1989; Wrather et al., 1995). Planting later also can help in avoiding cool, wet soil conditions conducive to SDS (Von Qualen et al., 1989; Wrather et al., 1995).

Though cultural methods can decrease SDS incidence and severity, resistant cultivars have provided the best management of SDS. Some *H. glycines*-resistant cultivars show tolerance to SDS (Hershman et al., 1990; Njiti et al., 1997; Rupe et al., 1991), while others have demonstrated severe susceptibility to SDS (Njiti et al., 1997). This demonstrates that while an interaction between *H. glycines* and Fv exists, cultivar resistance to both pathogens is not necessarily linked and cultivar selection should be based on resistance to SDS, not only resistance to SCN (Njiti et al., 1997). Cultivars with resistance to SDS show reduced foliar symptoms and may or may not slow root colonization by the fungus (Luo et al., 1999). These resistant cultivars are only partially resistant, but the partial resistance is highly heritable (Hnetkovsky et al., 1996; Iqbal et al., 2002). Many SDS-resistant lines have been identified. Hartman et al. (1997) screened over 800 soybean plant introductions, many from China, and found varying levels of resistance. Mueller et al. (2002) screened 6,037 plant inductions for resistance to SDS, finding 57 PIs resistant to SDS and suitable for resistance breeding. In addition, mapping efforts in several soybean cultivars have resulted in identification of several QTL for SDS-resistance that may be used in resistance breeding (Iqbal et al., 2002; de Farias Neto, 2007).

Biological methods of control for SDS may also be possible. Although no published reports of biological control of the SDS pathogen is available, some scientists have evaluated biological control agents for their efficacy against other *Fusarium* species. Cubeta et al. (1985) tested *Bacillus subtilis* for antagonism against twenty-six fungi associated with soybean seed diseases. In this study, it was determined the bacterial by-products were effective in slowing fungal development and could prove useful in controlling seedborne soybean pathogens (Cubeta et al., 1985). Similarly, *Bacillus pumilus* produces antifungal metabolites, which inhibit mycelial growth of many species of *Aspergillus*, *Penicillium*, and *Fusarium* (Munimbazi and Bullerman, 1998). Bradley (2008) reported that *B. pumilus* protected against stand and yield losses in soybean in North Dakota, but not as consistently as chemical seed treatments. Rojo et al. (2007) showed decreased brown root rot caused by *Fusarium solani* in peanut using different strains of *Trichoderma*.

Several chemical seed treatments for controlling soilborne seedling diseases of soybean have been identified. Poag et al. (2005) determined that soybean seed treatments costing less than \$8.65/ha averaged a return of \$43.71/ha due to protection against soilborne and seedborne pathogens in Arkansas. Other studies have shown mixed results with the use of fungicide seed treatments on soybean, with environment and pathogens present playing a role (Bradley, 2008; Bradley et al., 2001; Dorrance and McClure, 2001; Dorrance et al., 2003). Several fungicide seed treatments have proven effective against multiple *Fusarium* spp.; though, were not tested on Fv. Quinone outside inhibitor fungicides (QoIs, also known as strobilurins), such as azoxystrobin (Bartlett, 2002; Ramirez, 2004; Broders et al., 2007), pyraclostrobin (Ellis

et al., 2011), and trifloxystrobin (Chala et al, 2003), have been effective against *Fusarium* spp. Fungicides like thiophanate-methyl (Yoshida et al., 2008), prothioconazole (Paul et al., 2008), and fludioxonil (Hewitt, 1998; Broders et al., 2007; Ellis et al., 2011) also offer control of *Fusarium* spp.

Despite SDS being one of the major soilborne diseases of soybean in the U.S., no reports of the effect of fungicide seed treatments on SDS disease have been published to date. New research has demonstrated that early infection is crucial in SDS disease development, sparking more interest in using fungicide seed treatments to control SDS. The objective of this study was to determine the efficacy of fungicide seed treatments on Fv and SDS development.

## MATERIALS AND METHODS

Trials were conducted in the field, greenhouse, and laboratory to evaluate the effect of seed treatments on Fv and development of SDS.

### Valmeyer, IL Field Experiment

**General trial information.** A field trial was conducted at Valmeyer, IL (southern Illinois) in 2008. The field was center-pivot irrigated, and naturally infested with Fv with a history of SDS. The trial was planted 1 May 2008, and plots were 4 rows wide (76 cm row spacing) and 3 m long at a population of 44.5 seeds/m<sup>2</sup>. The previous crop was corn. For weed control, glyphosate at 0.95 kg a.e./ha + S-metolachlor at 1.26 kg a.i./ha (Sequence, Syngenta Crop Protection, Greensboro, NC) was applied 41 days after planting (DAP). Irrigation began at soybean growth stage R1 (Fehr et al., 1971) at a rate of 7.6 x 10<sup>5</sup> liters/ha each week until soybean plants were mature. The treatment design was a 2 cultivar x 12 seed treatment factorial arranged in a randomized complete block design (RCBD) with 4 replications.

**Soybean cultivars.** Cultivars Pioneer 94M30 (4.3 relative maturity, RM) and FS HiSoy 4456 (4.4 RM) were planted. Cv. Pioneer 94M30 was considered to be moderately resistant (MR) to SDS, and cv. FS HiSoy 4456 was considered to be moderately susceptible (MS) to SDS based on evaluations from the seed companies and the University of Illinois's Variety Information Program for Soybeans ([www.vipsoybeans.org](http://www.vipsoybeans.org)).

**Seed treatments.** No commercial fungicide seed treatments have been labeled for control of Fv; however, multiple fungicides have shown effective control of other

*Fusarium* species. Several fungicides utilizing different modes of action, active ingredients, and chemistries, were used in various combinations and tested. Mefenoxam (Apron XL, Syngenta Crop Protection) or metalaxyl (Allegiance FL, Bayer CropScience, Research Triangle Park, NC) were used in this study to limit seedling diseases caused by oomycete pathogens (Hewitt, 1998; Uesugi, 1998). The other fungicides tested were: fludioxonil (Maxim 4 FS, Syngenta Crop Protection); azoxystrobin (Dynasty, Syngenta Crop Protection); trifloxystrobin + metalaxyl (Trilex AL, Bayer CropScience); prothioconazole (Redigo, Bayer CropScience); *Bacillus pumilus* GB34 (Yield Shield, Bayer CropScience); thiophanate-methyl (Topsin 4.5 FL, United Phosphorus Inc., King of Prussia, PA); and thiophanate-methyl + pyraclostrobin (BAS 580, BASF Corporation, Research Triangle Park, NC) in multiple combinations. Non-treated seed of the cultivars also were included as a treatment. Non-treated seed and the mefenoxam only treated seed were considered controls. Seed treatment mixtures and application rates (g a.i./100 kg seed) are listed in Table 1.

**Root evaluations.** Eight to twelve plants were dug from one of the outside rows of each plot and taken back to the laboratory at 41 DAP, 55 DAP, and 70 DAP (soybean developmental/reproductive stages V4, V7 [R2], and V12 [R4], respectively). In the laboratory, the roots were soaked in water for 24 hours to remove soil. The plant shoot (tissue above the soil line) was cut off and discarded, and the root system (tissue below the soil line) was retained. Three roots systems from each plot were placed in a clear plastic tray, immersed in water, and digitally scanned with a flatbed scanner (Epson Expression 10000XL, Epson American, Inc., Long Beach, CA) at 400 dpi with a pixel size of 0.063 mm, and root length, root surface area, average root diameter, root volume,

number of root tips, and number of root forks or branches were evaluated using specialized software (WinRHIZO Pro2007d, Regent Instruments Inc., Quebec, Canada) following procedures described by Ortiz et al. (2004). A mean value of each measurement was calculated for each plot.

**Disease evaluations.** Beginning 110 DAP, SDS incidence and severity were rated weekly for four consecutive weeks. The percentage of plants with SDS symptoms was estimated for each plot (% incidence), and the average plot severity (% leaf area showing chlorotic and necrotic symptoms) also was estimated. A percent disease index (DX) was calculated as:  $DX = [\% \text{ incidence} \times \% \text{ severity}]/100$ . The area under disease progress curve (AUDPC) was calculated as:  $\sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = DX at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations. AUDPC was calculated using the first two DX scores, due to premature defoliation caused by SDS and senescence due to soybean maturity resulting in inaccurate DX scores in the last two foliar ratings.

**Plant stand, grain yield, and 100-seed weight.** At 41 DAP, plant stand was evaluated by counting the number of emerged plants in a 4 m row length in both middle rows of each plot, and then converting this to plants per  $m^2$ . Plots were harvested with a small plot combine on 9 October 2008 and yields were determined and adjusted to 13.5% moisture. Harvested seed samples from each plot were collected and taken back to the laboratory. One-hundred seeds were counted from each sample and weighed to determine 100-seed weight for each plot. Percent seed moisture was determined with a Burrows Digital Moisture Computer 700 (Seedburo Equipment Co., Des Plaines, IL).



**Fv DNA analysis in roots.** The roots collected at V4 not used for root scan analyses were used for a quantitative polymerase chain reaction (qPCR) assay to determine the relative amount of Fv DNA present in root tissue. Roots were surface-sterilized in a 0.5% NaOCl solution for 90 seconds, rinsed twice in sterilized distilled water, and stored at -80°C for later analysis. Frozen roots were then freeze-dried (VirTis General Purpose & Floral Freeze Dryer, SP Industries, Gardiner, NY) (Gao et al., 2004). Lateral roots were cut from the taproots, and taproots were ground with a Thomas Wiley Mini-Mill (Arthur H. Thomas Co., Swedesboro, NJ) until the grounds would pass through a 20-mesh filter. DNA was extracted from 50 mg of the ground root tissue using the FastDNA Spin Kit (MP Biomedicals, Solon, OH) following the manufacturer's protocol with amendments to remove DNA amplification inhibitors, as described by Malvick and Grunden (2005). Sample tubes containing one bead, garnet and root tissue received 1.2 mls of cell lysis solution-yeast (CLS-Y) extraction buffer, 0.33% polyvinylpyrrolidone (PVP) (Sigma-Aldrich Corp., St. Louis, MO), and a second bead. Samples were homogenized using a Fastprep - 24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH) twice for 30 s at a 4.5 speed setting. Samples were then centrifuged at 12,200 relative centrifugal force (rcf) and supernatant was collected in a new tube. This process was repeated and the supernatant was mixed with an equal amount of binding matrix. The solution was centrifuged and the pellet was re-suspended in 500 µl of ethanol wash solution (SEWS-M). The binding matrix was placed in the spin module with a catch tube and centrifuged twice at 12,200 rcf for 60 s. The DNA was then eluted in the spin module by re-suspending the pellet in 100 µl of DNase free water (DES) and incubated for 10 min at 40°C. The binding matrix was then centrifuged

for 1 min at 12,200 rcf and the DNA was collected in the recovery tube, and stored at -80°C.

Once the extraction was complete, DNA samples were prepared for the qPCR assay. DNA was diluted to a 10% concentration in molecular-grade distilled water (MP Biomedicals) and non-acidilated bovine serum albumin (BSA) at 400ng/μl (Fisher Scientific, Pittsburgh, PA) was added to samples, to further suppress DNA amplification inhibition (Jiang et al., 2005). Taqman primer and probes developed by Li et al. (2008) were used for the qPCR protocol (synthesized by Applied Biosystems, Foster City, CA). A synthetic DNA sequence was run with the sample as an internal control to identify false negatives, as described by Haudenshield and Hartman (2011). Samples were run in duplicate in 96-well qPCR plates and DNA amplification was done with a Stratagene Mx3005P RT-PCR (Agilent Technologies, Inc., Santa Clara, CA) machine. Thermal cycling conditions were as follows: 50°C for 120 s, 95°C for 120 s, then 40 cycles of 95°C for 15 s and 60°C for 60 s (Haudenshield and Hartman, 2011). Picograms of Fv DNA per mg of root tissue was determined using a standard curve created from a dilution series (1,000, 100, 10, 1, and 0.1 pg/μl) of pure Fv DNA included in each plate. Pure Fv DNA was obtained from pure Fv isolate Mont-1/FSG1, originated from Monticello, IL (Farias Neto et al., 2006; Hartman et al., 1997), grown in potato dextrose broth (Difco Laboratories, Detroit, MI) following procedures used by Gao et al. (2004) and the DNA extraction protocol described above. DNA was further purified using an E.Z.N.A. MicroElute DNA Clean-Up Kit (Omega Bio-Tek, Inc., Norcross, GA) and concentration determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE).

**Statistical analyses.** Data were analyzed using SAS software (version 9.2, SAS Institute Inc., Cary, NC) using the mixed models procedure (PROC MIXED) to make estimates comparing fixed effects. Cultivar and seed treatment were considered fixed effects. Block was considered a random effect. Residuals were examined with the Shapiro-Wilk test for normality ( $\alpha = 0.01$ ) using the univariate procedure (PROC UNIVARIATE) and Brown-Forsythe test for homogeneity of variance ( $\alpha = 0.05$ ) using the general linear model procedure (PROC GLM). Natural log transformation was required to normalize qPCR data for analysis. Estimate statements were used to compare treatment least-square means (LS-means). Correlations between Fv DNA concentration in roots and SDS AUDPC values were determined using Pearson correlation procedure (PROC CORR) in SAS (version 9.2). Terms, estimates, and correlations were considered significant at  $P \leq 0.05$

### **Urbana, IL Field Experiment**

**General trial information.** Field trials were conducted at Urbana, IL (central Illinois) in 2008 and 2009. The trial was planted 22 May 2008 and 29 May 2009, and plots were 4 rows wide (76 cm row spacing) and 4.6 m long at a population of 44.5 seeds/m<sup>2</sup>. The previous crop was soybean. The soil was naturally infested with Fv and augmented with sterilized sorghum seed infested with Fv prior to planting soybeans. To augment the soil with Fv, the sterilized sorghum seed infested with Fv (inoculum) was prepared (procedure described below). Just prior to planting the soybeans, the sorghum inoculum was planted with the planter using the deepest setting (approximately 10 cm). Approximately 0.14 kg of sorghum inoculum was planted into each 4-row plot. The

planter was then set at a depth of 3 cm, and soybean seeds were planted directly over the sorghum inoculum as described by Farias Neto et al. (2006). For weed control, dimethenamid-P at 0.95 kg a.i./ha (Outlook, BASF Corporation) and imazethapyr at 0.07 kg a.i./ha (Pursuit, BASF Corporation) was applied pre-emergence, 25 May 2008 and 23 May 2009. A post-emergence herbicide application was made with glyphosate at 1.17 kg a.e./ha (Touchdown, Syngenta Crop Protection) 20 June 2008 and 22 June 2009. Plots were not irrigated in 2008, but were drip-tape irrigated in 2009. Irrigation in 2009 began 12 DAP and soil was maintained at near field capacity until plants were mature. The treatment design was a 2 cultivar x 12 seed treatment factorial arranged in a RCBD with 4 replications.

**Inoculum preparation.** Fv inoculum was prepared for the field by soaking white sorghum [*Sorghum bicolor* (L.) Moench] seeds in water for 16 hours. Water was drained, and 4 kg of sorghum seed were placed in each 61 x 91 cm autoclave bag (Fisher Scientific), and autoclaved on consecutive days at 121°C for 60 mins. PDA from a single plate, fully colonized by Fv isolate Mont-1 (Farias Neto et al., 2006), was cut into multiple sections and added to the sterilized sorghum seeds after cooling each bag. A sterilized foam plug (Fisher Scientific) was placed in the opening of each bag and zip-tied in place to allow air circulation and limit contamination. Bags were shaken daily to mix the Fv and sorghum seeds. The inoculum was air dried after incubating 2 weeks at 24°C (Farias Neto et. al., 2006). Once dry, inoculum was placed in sewn paper bags and stored in a 4°C walk-in cooler.

**Soybean cultivars.** Cultivars HiSoy 3466 (3.4 RM) and NK 33-A8 (3.3 RM) were planted. Cv. HiSoy 3466 was considered to be moderately resistant (MR) to SDS,

and cv. NK 33-A8 was considered to be moderately susceptible (MS) to SDS based on evaluations from the seed companies and the University of Illinois's Variety Information Program for Soybeans ([www.vipsoybeans.org](http://www.vipsoybeans.org)).

**Seed treatments.** Seed treatments were the same as those used for the Valmeyer field trial. Non-treated seed and the mefenoxam only treated seed were considered controls. Table 1 lists all seed treatments and application rates.

**Root evaluations.** Eight to twelve plants were dug from one of the outside rows of each plot and taken back to the laboratory at 22 DAP, 36 DAP, and 51 DAP (soybean developmental/reproductive stages V1, V3, and V6 [R1], respectively) in 2008. In 2009, plants were dug at 20 DAP, 37 DAP, and 51 DAP (soybean developmental stages V1, V3, and V6 [R1], respectively) in 2009. In the laboratory, root systems were prepared and analyzed following the same protocol used for the Valmeyer field trial described above.

**Disease evaluations.** SDS incidence and severity were rated weekly for four consecutive weeks. In 2008, no SDS foliar symptoms were present at any time in the trial. Plots in 2009 were rated beginning 83 DAP. DX and AUDPC were calculated for 2009, as described above for the Valmeyer field trial. All DX scores collected at the four timings in 2009 were used to calculate AUDPC.

**Plant stand, grain yield, and 100-seed weight.** In 2008 and 2009, plant stand was evaluated at 22 DAP and 20 DAP respectively, by counting emerged plants in a 4 m row length in both middle rows of plots and calculating plants per m<sup>2</sup>, as described for the Valmeyer field trial. Plots were harvested with a small plot combine on 26 September 2008 and 19 October 2009 to determined yields adjusted to 13.5% moisture.

Grain samples were measured to determine percent moisture and 100-seed weight for each plot as described above for the Valmeyer field trial.

**Fv DNA analysis in roots.** The roots collected at V1 from 2008 and 2009 were used for the qPCR assay as described above for the Valmeyer field trial.

**Statistical analysis.** Data were analyzed with SAS software (version 9.2, SAS Institute Inc.) following the procedure described for the Valmeyer location. Year and blocks nested within years were considered random effects. Natural log transformation was used for qPCR data, 22 DAP and 36 DAP average root diameters, and 22 DAP and 36 DAP root tip counts. Sine transformation was used for seed moisture. Square root transformation was used for 22 DAP, 36 DAP, and 51 DAP root fork counts, 36 DAP and 51 DAP root volumes, and 51 DAP root tip counts. Data from the 2008 and 2009 were pooled for analysis and Best Linear Unbiased Predictions (BLUPs) were used to compare LS-means of random effects. Estimates and BLUPs were considered significant at  $P \leq 0.05$ .

### **Greenhouse Experiment**

**General trial information.** The design of the greenhouse experiment was a 3 cultivar x 12 seed treatment factorial with 3 replications arranged in a complete randomized design (CRD). The experiment was repeated once. Paper towels were placed in the bottom of 10 x 35 x 50 cm trays (Hummert's Own Dyna-flat, Hummert International Horticultural Supplier, Earth City, MO) with drainage holes to prevent soil loss. A steam-pasteurized sand:soil (2:1) mixture was placed into each tray and leveled (approximately 4 liters of sand:soil mixture per tray). A wooden template was pressed

into the soil to create 8 rows with 5.6 cm row spacing, 2 cm deep, the width (35 cm) of the tray. Fv sorghum inoculum was prepared as described above, and approximately 10 cm<sup>3</sup> of sorghum inoculum was evenly distributed in each furrow, and were covered with approximately 2 cm of the sand:soil mixture. The template was then used to create 0.5-cm-deep furrows directly over the top of the inoculum. Each furrow was evenly divided into 2, 10-seed plots with a 5 cm space between plots. Growing conditions throughout the trial were controlled for a 16-h photoperiod, approximately 25°C air temperature, and the soil was maintained at a near field capacity (Mueller et al., 2003).

**Soybean cultivars.** Cultivars Pioneer 94M30 (4.3 RM), HiSoy 3466 (3.4 relative maturity, RM) and NK 33-A8 (3.3 RM) were used for the greenhouse trial. Cv. HiSoy 3466 and Pioneer 94M30 were considered to be MR to SDS, while NK33-A8 was considered MS to SDS.

**Seed treatments.** Cultivars received the same seed treatments and rates as in the field experiments (Table 1). Due to steam treated soil and the controlled conditions of the greenhouse, there was less concern of oomycete pathogens interfering with results. Therefore, the non-treated seed acted as the control in the greenhouse experiment.

**Disease evaluations.** SDS foliar symptoms were rated six times beginning at initial disease development (14 DAP in the first trial and 12 DAP in the second trial) and approximately every 3 days afterwards. Disease incidence was calculated as: % incidence = (number of plants showing SDS symptoms/stand count) x 100. Due to the increased defoliation and plant death in the greenhouse compared to the field trials, a 1-to-7 scale was used to rate disease severity, where 1 = leaves showing general yellowing

and /or blotches, 2 = leaves with obvious, interveinal chlorosis, 3 = leaves with necrosis along a portion of the margin, 4 = necrosis along the entire leaf margin, 5 = interveinal necrosis and more than 50% of leaf tissue is necrotic, 6 = most of leaf area is necrotic and plants are defoliated with new growth, and 7 = dead and defoliated plants (adjusted scale from C. R. Bowen and T. Lynch-Slaminko, [www.vipsoybean.org](http://www.vipsoybean.org)). DX was calculated as:  $DX = \{ \% \text{ incidence} \times [(\text{severity}/7) \times 100] \} / 100$ . AUDPC was calculated as described above for the field experiments.

**Root evaluations.** All roots from each plot were collected at the conclusion of the trials, 35 DAP. Three roots from each plot were scanned and analyzed with root analysis software as described for the Valmeyer field study. The plot means for root length, root surface area, average root diameter, root volume, number of root tips, and number of root forks were calculated.

**Fv DNA analysis in roots.** All roots collected from greenhouse plots were processed following the protocols described for the Valmeyer field experiment, and the qPCR assay was conducted as described above for the Valmeyer field experiment. Picograms of Fv DNA per mg of root tissue was calculated using the standard curve created from the pure Fv DNA dilution series run with all greenhouse root samples.

**Statistical analysis.** Data were analyzed using SAS software (version 9.2, SAS Institute Inc.) following procedures listed above. Data from both trials were pooled for analysis. Trial was considered a random effect. Root length, surface area, tip count, and fork count data were square root transformed for analysis. A natural log transformation



was used for average root diameter. Estimates and BLUPs, used to compare LS-means, were considered significant at  $P \leq 0.05$ .

### **Laboratory Experiment**

**Rolled-towel assay.** A paper towel assay similar to that described by Ellis et al. (2011) was used to evaluate the effects of fungicide seed treatments on Fv-infested soybean seeds. The assay consisted of 14 seed treatments with 4 replications arranged in a CRD repeated twice over time. Cultivar Pioneer 92Y80 was treated with one of the 14 seed treatments and 10 seeds of each treatment were placed in a row down the center along the length of a sterilized paper towel (30 cm x 45 cm) moistened with sterilized, distilled water. Inoculated seeds had a 100 µl suspension of Fv macroconidia at a concentration of  $2.5 \times 10^5$  macroconidia/ml applied directly to the seed with a micropipette. A second moistened paper towel was placed over the seeds and the towels were rolled perpendicularly to the seed row. Treatments were then placed in partially-sealed plastic bags and set up-right in growth chambers. Growth chambers were maintained at 23°C with the lights turned off, and paper towels were kept moist with sterilized, distilled water for 14 days.

**Inoculum preparation.** Fv isolate Mont-1 was grown on PDA for 3 to 4 weeks until sporulation was observed. Macroconidia were dislodged from the agar surface with a sterilized glass rod into 3 to 5 ml of sterilized, distilled water, and then transferred with a pipette and filtered through 3 layers of cheese cloth to remove mycelial fragments (Ellis et al., 2011). The macroconidial suspension was quantified using a hemacytometer (Bright-Line Hemacytometer, Hausser Scientific, Horsham, PA) as described by Tuite

(1969). Sterilized distilled water was added to the suspension to achieve a concentration of  $2.5 \times 10^5$  macroconidia/ml.

**Soybean cultivar.** Cultivar Pioneer 92Y80 (2.8 RM) was used for the rolled-towel assay. Cultivar Pioneer 92Y80 was considered moderately resistant to SDS by the seed company.

**Seed treatments.** The same active ingredients from the field and greenhouse experiments were used in the rolled-towel assay; however, modifications to treatments were made. Active ingredients were tested individually, with fewer fungicide combinations. Several treatments also were added, including: non-treated, non-inoculated seed; NaOCl surface-sterilized, non-inoculated seed; and NaOCl surface-sterilized, inoculated seed. Seeds were soaked in a 0.5% NaOCl solution for 90 s and rinsed twice with sterile water to limit seed borne diseases. The NaOCl surface sterilized, inoculated seeds acted as the control. The treatment list and application rates are listed in Table 2.

**Plant evaluations.** After 14 days, total plant length and lesion length were measured for each plant using a ruler. A disease severity index was calculated by dividing lesion length by plant length and multiplying by 100. Seeds were considered non-germinated if the radicle length was not greater than two times the seed length. Non-germinated seeds colonized by Fv were given an index rating of 100% (Ellis et al. 2011). Percent germination was calculated as well.

**Statistical analysis.** Data were analyzed using SAS software (version 9.2, SAS Institute Inc., Cary, NC) as described above. Data from trials were pooled for analysis.

Seed treatment was considered a fixed effect, while trial was considered a random effect. Germination data was square root transformed, while a log transformation was utilized for lesion length and disease index. Estimates and BLUPs, used to compare LS-means, were considered significant at  $P \leq 0.05$ .

## RESULTS

### Valmeyer, IL Field Experiment

Based on the analysis, the main effects of cultivar and seed treatment were significant for plant stand and SDS (Table 3). The main effect of cultivar was significant for 100-seed weight and yield, and the cultivar x seed treatment interaction was significant for 100-seed weight. No effects were significant for Fv DNA or seed moisture. For the V4 root collection timing, the main effect of cultivar was significant for root length, root surface area, root volume, and root forks (Table 4). At this same timing, the main effect of seed treatment was significant for root volume only, and the interactive cultivar x seed treatment effect was significant for root length only. For the V7 root collection timing, the main effect of cultivar was significant for root length, average root diameter, and root forks. At the same timing, the main effect of seed treatment and the interactive effects of cultivar x seed treatment were significant for average root diameter and root tips. For the V12 root collection timing, no main effects or interactive effects were significant. Main effect means are presented and discussed below only when they did not interact with other factors; otherwise, the interactive effect means are presented and discussed below.

Cultivar FS 4456 had a greater plant stand ( $32.9 \text{ plants/m}^2$ ) than cv. Pioneer 94M30 ( $27.5 \text{ plants/m}^2$ ). All treatments except metalaxyl + prothioconazole + trifloxystrobin + *B. pumilus* and mefenoxam + azoxystrobin + thiophanate-methyl + *B. pumilus* + prothioconazole + fludioxonil had greater plant stands than the non-treated control and the mefenoxam only control (Table 5).

Cultivar FS 4456 had a greater SDS AUDPC value (AUDPC = 265) than cv. Pioneer 94M30 (AUDPC = 102). Only mefenoxam + azoxystrobin had a lower SDS AUDPC value than the mefenoxam only control (Table 5). All treatments except metalaxyl + prothioconazole + trifloxystrobin had a lower SDS AUDPC values than the non-treated control. The correlation between Fv DNA concentration in soybean roots and SDS AUDPC values were not significant ( $R = 0.12$ ,  $P = 0.2776$ ).

Cultivar Pioneer 94M30 had a greater yield (5,046 kg/ha) than cv. FS 4456 (4,092 kg/ha). For cv. FS 4456, no treatments had significantly different 100-seed weights than the mefenoxam only control, but metalaxyl + prothioconazole + trifloxystrobin and mefenoxam + azoxystrobin + thiophanate-methyl + *B. pumilus* + prothioconazole + fludioxonil had lower 100-seed weights than the non-treated control (Table 6). For cv. Pioneer 94M30, metalaxyl + prothioconazole + trifloxystrobin and mefenoxam + thiophanate-methyl had higher 100-seed weights than both the non-treated control and the mefenoxam only control.

For roots collected at V4, no treatments were significantly different than the non-treated control or the mefenoxam only control for root length for cv. FS 4456 (Table 7). For cv. Pioneer 94M30, mefenoxam + fludioxonil, metalaxyl + prothioconazole + trifloxystrobin, metalaxyl + prothioconazole + trifloxystrobin + *B. pumilus*, mefenoxam + thiophanate-methyl, and mefenoxam + azoxystrobin + thiophanate-methyl + *B. pumilus* + prothioconazole + fludioxonil had smaller root lengths than the non-treated control. No treatments were significantly different than the mefenoxam only control for root length.

For roots collected at V4, cv. Pioneer 94M30 had a greater root surface area (28.4 cm<sup>2</sup>) than cv. FS 4456 (25.0 cm<sup>2</sup>). Cultivar Pioneer 94M30 also had a greater root volume (0.5 cm<sup>3</sup>) and number of root forks (368 forks) than cv. FS 4456 (root volume = 0.4 cm<sup>3</sup>; 321 forks).

For roots collected at V7, cv. Pioneer 94M30 had a greater root length (212 cm) than cv. FS 4456 (196 cm). Cultivar Pioneer 94M30 also had a greater number of root forks (680 forks) than cv. FS 4456 (617 forks). For cv. FS 4456, only mefenoxam + azoxystrobin + fludioxonil had a significantly lower average root diameter than the mefenoxam only control, and no treatments were significantly different than the non-treated control for average root diameter (Table 8). For cv. Pioneer 94M30, all treatments except mefenoxam only, mefenoxam + fludioxonil, and mefenoxam + fludioxonil + azoxystrobin had significantly smaller average root diameters than the non-treated control, and these same treatments and the non-treated control and metalaxyl + trifloxystrobin were the only treatments that did not have smaller average root diameters than the metalaxyl only control.

For roots collected at V7, only mefenoxam + fludioxonil was different than the mefenoxam only control and had significantly fewer root tips than the mefenoxam only control for cv. FS 4456. No treatments were different than the non-treated control for number of root tips in cv. FS 4456 (Table 8). For cv. 94M30, only metalaxyl + prothioconazole + trifloxystrobin, metalaxyl + prothioconazole + trifloxystrobin + *B. pumilus*, and mefenoxam + thiophanate-methyl were different than the non-treated control and had greater numbers of root tips. Only metalaxyl + prothioconazole +

trifloxystrobin + *B. pumilus* and mefenoxam + thiophanate-methyl were significantly different than the metalaxyl only control and had greater numbers of root tips.

### **Urbana, IL Field Experiment**

According to PROC MIXED, the year x cultivar interaction effect was significant for plant stand, yield, 100-seed weight, and seed moisture (Table 9). The main effect of cultivar was significant for SDS in 2009 (no SDS was observed in 2008). No effects were significant for Fv DNA. At the V1 root collection timing, cultivar main effect was significant for root length and root surface area (Table 10). The cultivar x seed treatment interaction effect was significant for root volume at this same timing. Also at this timing, the year main effect was significant for root tips. A significant year x cultivar interaction effect was observed for number of tips at the V1 root collection timing. No effects were significant for average root diameter and root forks at the V1 timing. For the V3 root collection timing, year main effect was significant for root length, root surface area, root tips, and root forks. At this same timing, year x cultivar interaction effect was significant for root length, root volume, root tips, and root forks. Cultivar main effects were significant for root surface area. The year x seed treatment interaction effect also was significant for root forks at the V3 timing. No effect was significant for average root diameter at V3. For the V6 collection timing, the year x cultivar interaction effect was significant for root length, root surface area, average root diameter, root volume, and root forks. The year main effect was significant for average root diameter and root tips at the V6 collection. Main effect means are presented and discussed below only when they did

not interact with other factors; otherwise, the interactive effect means are presented and discussed below.

In 2009, cv. FS 3466 had a lower SDS AUDPC value (AUDPC = 157) than cv. NK 33-A8 (AUDPC = 664). Within years, cultivars did not significantly differ in plant stands (Table 11). In general, the plant stands observed in 2009 were greater than those observed in 2008, which is why a significant year x cultivar interaction was observed for plant stand. No significant correlation was found between Fv DNA concentrations and SDS AUDPC ( $R = 0.12$ ,  $P = 0.2776$ ).

In 2008, cv. FS 3466 had a significantly lower yield than cv. NK 33-A8; however, in 2009, cv. FS 3466 had a significantly higher yield than cv. NK 33-A8 (Table 11). In 2008, 100-seed weight was not different for cultivars, but in 2009, cv. FS 3466 had a lower 100-seed weight compared to cv. NK 33-A8. Cultivar FS 3466 had higher seed moisture than cv. NK 33-A8, in 2008; however, cv. FS 3466 had significantly lower seed moisture than cv. NK 33-A8 in the following year.

At the V1 root collection timing, cv. FS 3466 had a greater root length (54.0 cm) than cv. NK 33-A8 (46.4 cm) both years. Cultivar FS 3466 had greater root surface area (15.99 cm) than c. NK 33-A8 (14.03 cm) across years. In 2008, cv. FS 3466 had a greater number of root tips (171 tips) than cv. NK 33-A8 (134 tips); however in 2009, the number of root tips counts were greatly reduced across cultivars and no significant difference was observed between cv. FS 3466 (45 tips) and cv. NK33-A8 (41 tips).

For roots collected at the V1 developmental stage, only metalaxyl + prothioconazole had a significantly greater root volume than the mefenoxam only control



for cv. FS 3466 (Table 12). No seed treatments had significantly different root volumes than the non-treated control for cv. FS 3466. For cv. NK 33-A8, no seed treatments had a significantly greater root volume than the mefenoxam only control, but several treatments had a significantly smaller root volume. Treatments that had significantly greater root volumes than the non-treated control for cv. NK 33-A8 were the mefenoxam only control, mefenoxam + azoxystrobin, metalaxyl + prothioconazole + trifloxystrobin, mefenoxam + thiophanate-methyl, and metalaxyl + pyraclostrobin + thiophanate-methyl.

For the V3 root collection timing, root surface area in 2008 ( $28.5 \text{ cm}^3$ ) was significantly greater than the root surface area in 2009 ( $13.9 \text{ cm}^3$ ). Across years, cv. FS 3466 had a greater root surface area ( $22.4 \text{ cm}^3$ ) than cv. NK 33-A8 ( $20.0 \text{ cm}^3$ ). At the V3 root collection timing in 2008, cv. FS 3466 had greater root length than NK 33-A8 (Table 13). In 2009, cultivars did not significantly differ for root lengths.

For roots collected at V3 in 2008, cultivars did not differ in root volumes; however, in 2009, cv. FS 3466 had a greater root volume than cv. NK 33-A8 (Table 13). The number of root tips was significantly greater for cv. FS 3466 compared to cv. NK 33-A8 in both 2008 and 2009. In 2008, cv. FS 3466 had more root forks than cv. NK 33-A8, but no differences in root forks were observed between cultivars in 2009. No significant differences among seed treatments within each year were observed for root forks (Table 14), but in general, more root forks were observed in 2008 than 2009.

At the V6 root collection timing, significantly more root tips were observed in 2008 (789 tips) than 2009 (237 tips). Roots from cv. FS 3466 were significantly longer than cv. NK 33-A8 in both 2008 and 2009 (Table 15). Cultivar FS 3466 had significantly

greater root surface area than cv. NK 33-A8 in 2008, but no significant difference was observed between the two cultivars in 2009. In 2008, soybean cultivars did not significantly differ for average root diameter, but cv. FS 3466 had a significantly smaller average root diameter compared to cv. NK 33-A8 in 2009. No significant difference between cultivar root volumes was observed within each year. In both 2008 and 2009, cv. FS 3466 had a greater number of root forks than cv. NK 33-A8.

### **Greenhouse Experiment**

No significant differences were observed among the main effects of trial, cultivar, or seed treatment for plant stand or SDS, but a significant trial x cultivar interaction was observed for plant stand and SDS (Table 16). Significant differences between trials were observed for Fv DNA, and significant trial x cultivar and trial x seed treatment interactions were observed for Fv DNA. Significant differences between trials were observed for root length, and significant trial x cultivar trial x seed treatment interactions were observed for root length. No differences among main effects were observed for root surface area, but a significant trial x cultivar interaction was observed for root surface area. Significant differences between trials were observed for average root diameter, and significant trial x cultivar and trial x seed treatment interactions were observed for average root diameter. No significant main effects or interactions were observed for root volume. For root tips, the main effects of trial and cultivar were significant. For root forks, the main effect of trial and the interactive effects of trial x cultivar and trial x seed treatment were significant. Main effect means are presented and discussed below only

when they did not interact with other factors; otherwise, the interactive effect means are presented and discussed below.

In trial 1, cv. NK 33-A8 had a greater plant stand than the other two cultivars, and cv. Pioneer 94M30 had a lower plant stand than the other two cultivars (Table 17). Cultivars did not significantly differ in plant stand for trial 2.

In trial 1, cv. NK 33-A8 had a greater amount of Fv DNA than cv. FS 3466 and cv. Pioneer 94M30 (Table 17). Cultivars did not significantly differ from each other for the amount of Fv DNA in trial 2. In both trial 1 and 2, the treatment containing mefenoxam + thiophanate-methyl + azoxystrobin + *B. pumilus* + prothioconazole + fludioxonil was the only treatment that had significantly less Fv DNA than the non-treated control (Table 18).

In trial 1, cv. NK 33-A8 had a greater SDS AUDPC value than cv. FS 3466 and cv. Pioneer 94M30 (Table 17). In trial 2, cv. Pioneer 94M30 had a greater SDS AUDPC value than cv. FS 3466 and cv. NK 33-A8. A significant positive correlation existed between Fv DNA concentrations in roots and SDS AUDPC values ( $R = 0.62$ ,  $P = <0.0001$ ).

There were no significant differences in root length among cultivars within trial 1 or within trial 2, however cultivars in trial 1 had longer root lengths than cultivars in trial 2 (Table 17). Similarly, no significant differences in seed treatment root lengths were observed within trials. Root lengths across all seed treatments were shorter in trial 2, except for the mefenoxam + thiophanate-methyl + azoxystrobin + *B. pumilus* + prothioconazole + fludioxonil treatment (Table 18).

In trial 1, cv. NK 33-A8 had less root surface area than the other two cultivars (Table 17). Cultivars did not significantly differ in root surface area in trial 2.

In trial 1, cv. FS 3466 had a smaller average root diameter than cv. Pioneer 94M30, but was not significantly different than cv. NK 33-A8. In trial 2, cv. FS 3466 had a smaller average root diameter than cv. NK 33-A8, but was not significantly different than cv. Pioneer 94M30. Average root diameters among seed treatments were not significantly different within trials. All seed treatments had smaller average root diameters in trial 1 than in trial 2 (Table 18).

Averaged over all treatments, the number of root tips was greater in trial 1 (393 tips) compared to trial 2 (128 tips). Cultivar NK 33-A8 had fewer root tips (234 tips) than cv. FS 3466 (294 tips) or cv. Pioneer 94M30 (289 tips).

The number of root forks each cultivar had was not significantly different within each trial, but the number of root forks for all three cultivars greatly decreased in trial 2 (Table 17). Seed treatments did not significantly differ in number of root forks within each trial, but in trial 2, the number of forks across all seed treatments was lower, with the exception of the mefenoxam + thiophanate-methyl + azoxystrobin + *B. pumilus* + prothioconazole + fludioxonil treatment (Table 18).

### **Laboratory Experiment**

According to PROC MIXED, a significant ( $P \leq 0.05$ ) difference between trials was observed for plant length (Table 19). Seed treatments significantly affected seed

germination, plant length, lesion length, and disease severity. Significant trial x seed treatment interactions were observed for plant length, lesion length, and disease severity.

Seed germination did not appear to be affected by Fv, since no differences were observed between non-treated non-inoculated seeds and the non-treated inoculated control. Seeds treated with *B. pumilus* had significantly lower seed germination than the non-inoculated, non-treated control (Table 20).

In general, plant length did not appear to be affected by Fv, as no differences in plant length were observed between non-treated seeds that were either inoculated with Fv or not inoculated with Fv in either trial 1 or trial 2 (Table 21). In Trial 1, no treatments had significantly larger plant lengths than the non-treated inoculated control; however, seeds treated with *B. pumilus* resulted into plants shorter than plants resulting from the non-treated inoculated control. In trial 2, only the NaOCl-treated, non-inoculated seeds had a greater plant length than the non-treated inoculated control. Seeds treated with *B. pumilus* or prothioconazole resulted into plants shorter than plants resulting from the non-treated inoculated control in trial 2.

Total lesion length did appear to be affected somewhat by Fv, in that NaOCl-treated seeds inoculated with Fv had increased lesion lengths compared to NaOCl-treated seeds not inoculated with Fv for both trials (Table 21). In addition, non-treated control seeds inoculated with Fv had increased lesion lengths compared to non-treated control seeds not inoculated with Fv for trial 2. For trial 1, seeds treated with fludioxonil, pyraclostrobin + thiophanate-methyl, thiophanate-methyl, or azoxystrobin + *B. pumilus* + fludioxonil + mefenoxam + prothioconazole + thiophanate-methyl had lesion lengths

smaller than the non-treated inoculated control. Seeds treated with NaOCl, azoxystrobin, prothioconazole, or pyraclostrobin had greater lesion lengths than the non-treated inoculated control treatment for trial 1. For trial 2, seeds treated with azoxystrobin, fludioxonil, pyraclostrobin, pyraclostrobin + thiophanate-methyl, thiophanate-methyl, or azoxystrobin + *B. pumilus* + fludioxonil + mefenoxam + prothioconazole + thiophanate-methyl had lesion lengths smaller than the non-treated inoculated control. No treatments had lesion lengths greater than the non-treated inoculated control in trial 2.

Disease severity did appear to be affected by Fv, in that NaOCl-treated seeds inoculated with Fv had increased disease severity compared to NaOCl-treated seeds not inoculated with Fv for both trials (Table 21). In addition, non-treated control seeds inoculated with Fv had increased disease severity compared to non-treated control seeds not inoculated with Fv for trial 2. In trials 1 and 2, seeds treated with fludioxonil or azoxystrobin + *B. pumilus* + fludioxonil + mefenoxam + prothioconazole + thiophanate-methyl had less disease severity than the non-treated inoculated control. Seeds treated with *B. pumilus* had greater disease severity than the non-treated inoculated control in trial 1. No treatments had greater disease severity than the non-treated inoculated control in trial 2.

## DISCUSSION

In this research, none of the seed treatments consistently demonstrated control of Fv or SDS. In greenhouse trials, the mefenoxam + thiophanate-methyl + azoxystrobin + *B. pumilus* + prothioconazole + fludioxonil treatment decreased the Fv DNA concentration in soybean roots; however, no seed treatments significantly decreased Fv DNA in field-grown soybean roots. SDS foliar symptoms were reduced by most seed treatments compared to the non-treated control at the Valmeyer, IL location. In addition, several seed treatments reduced lesion length and SDS disease severity compared to the non-treated inoculated control from the paper-towel assay; though, *B. pumilus* noticeably increased lesions and SDS severity and decreased germination and plant length compared to the control. No significant seed treatment effect was observed for SDS severity for the Urbana field or the greenhouse trials. A few seed treatments increased 100-seed weight of soybeans compared to the non-treated control within cultivars in Valmeyer, but no other seed treatment effects were observed across harvest data at either location.

Root scan analyses showed few significant seed treatment effects across field and greenhouse trials. At Valmeyer, IL, root lengths of Pioneer 94M30 were decreased by several seed treatments compared to the non-treated control at the V4 developmental growth stage, and depending on cultivar, seed treatments appeared to have both negative and positive effects on the V7 developmental stage average root diameter and number of tips compared to the control. Analyses of roots collected from Urbana, IL field trials resulted in significant cultivar by seed treatment effects and year by seed treatments effects for number of root tips at V1 and V3 soybean developmental stages, respectively.

Effects due to trial by seed treatment in the greenhouse often were the result of general shifts in means across treatments due to trial effects.

Differences between cultivars moderately resistance to SDS and cultivars moderately susceptible to SDS were significant across field and greenhouse trials. The moderately resistant cultivars tended to have healthier root systems, less SDS, and yielded better than the susceptible cultivars when disease was present in the field; though, cultivar resistance did not affect Fv root colonization. Interactions between years and cultivars were very common at the Urbana location and in the greenhouse. In both experiments, root rot and foliar disease were more prevalent in the second trial. In the second greenhouse trial, in some instances, cultivar resistance appeared to be overwhelmed as reported by Njiti et al. (2001). Few interactions between cultivars and seed treatments were observed.

The seed treatments selected for this trial had shown some effectiveness against *Fusarium* spp., except for metalaxyl and mefenoxam included in this study to control oomycete pathogens (Schwinn and Staub, 1995; Hewitt, 1998; Uesugi, 1998). Quinone outside inhibitor fungicides (QoIs, also known as strobilurins), such as azoxystrobin (Barlett, 2002; Ramirez, 2004; Broders et al., 2007), pyraclostrobin (Ellis et al., 2011), and trifloxystrobin (Chala et al, 2003), have shown activity against *Fusarium* spp. Thiophanate-methyl (Yoshida et al., 2008) is effective against *Fusarium* spp. and widely used to control *F. graminearum* in wheat. Fludioxonil also is effective against *Fusarium* spp., such as *Fusarium graminearum* (Hewitt, 1998; Broders et al., 2007; Ellis et al., 2011). *B. pumilus* has been shown to inhibit *Fusarium* mycelial growth, while inducing systemic resistance in plants and promoting plant growth (Munimbazi and Bullerman,



1998; Kloepper et al., 2004). Prothioconazole was selected because it has shown activity against *Fusarium* spp. (Paul et al., 2008). It is possible for fungicides to differ in efficacy against different *Fusarium* spp. (Munkvold et al., 2002). Munkvold et al. tested captan, difenoconazole, and fludioxonil on multiple isolates of *F. graminearum*, *F. verticillioides*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. subglutinans* and discovered the fungicides effectiveness varied not only by species but by isolates within a species, as well.

Studies have shown the benefit of fungicide seed treatments often depends on host susceptibility, environmental conditions, and the pathogens present (Bradley, 2008; Bradley et al., 2001; Dorrance and McClure, 2001; Dorrance et al., 2003). In the rolled-towel assay, *B. pumilus* decreased seed germination, as did several other treatments to a lesser extent. Since Fv is not known to affect seed germination, and the assay was designed so Fv was the only pathogen present, fungicides were not expected to benefit seed germination. Seed germination could have been reduced by seed treatments due to toxic effects on the germinating seeds by treatments or by treatments acting against beneficial microorganisms that may have been present on the seeds and aiding in seedling development (Raaijmakers et al., 2009).

Inconsistencies between greenhouse and field results have been a common obstacle when evaluating cultivars for SDS resistance and could be the result of significantly higher infection rates in greenhouse experiments compared to field experiments (Njiti et al., 2001). Similar inconsistencies were observed in this research. The greenhouse results showed that Fv DNA concentrations in roots were much higher than in the field trials. In fact, greenhouse Fv DNA concentrations were greater in trial 2

compared to trial 1, which may have overwhelmed the cultivar resistance. The reasons for differences in Fv concentrations among greenhouse trials are unclear but could have been the result of variation in inoculum.

There was no significant correlation between Fv DNA concentrations in roots and SDS AUDPC values measured in the field experiments; however a significant positive correlation was found in the greenhouse experiment. These results agree with the observations from the field. Years were not statistically different for Fv DNA concentrations in roots at Urbana, IL; though, SDS foliar symptoms were severe in 2009 but no disease was present in 2008. The severe disease was likely due to irrigation used in 2009 and not in 2008. This corresponds to results reported by Melgar et al. (1994) and Farias et al. (2006). A possible explanation for the difference between the field and greenhouse correlation results could be the root collection timing in relation to the presence of foliar symptoms. In the field trials, roots were collected for the Fv DNA concentration analysis several weeks before SDS symptom expression; conversely, greenhouse roots were collected upon completion of the trial when SDS symptoms were present.

Interest in using seed treatments to control SDS has increased recently. New research has shown that early root infection by Fv is critical for disease development (Navi and Yang, 2008; Gongora-Canul and Leandro, 2011). It may be possible for seed treatments to inhibit early infection thus limiting the disease later in the season; however, none of the seed treatments evaluated in this trial consistently inhibited Fv infection or sudden death syndrome in soybeans. In this research, cultivar resistance provided the best control for SDS and is one of the most effective management tools available to

producers (Roy et al, 1997). Perhaps, in the future, seed treatments will prove to be valuable management tools to control sudden death syndrome.

## TABLES

Table 1. The seed treatments and rates used in field and greenhouse experiments.

Seed treatment			grams a. i. per 100 kg seed
#	Trade name	Active ingredient	
1		non-treated	
2	Apron XL	mefenoxam	3.75
3	Maxim 4 FS	fludioxonil	5
	Apron XL	mefenoxam	3.75
4	Dynasty	azoxystrobin	3
	Apron XL	mefenoxam	3.75
5	Maxim 4 FS	fludioxonil	5
	Dynasty	azoxystrobin	3
	Apron XL	mefenoxam	3.75
6	Trilex AL	trifloxystrobin + metalaxyl	5 + 4
7	Redigo	prothioconazole	5
	Allegiance FL	metalaxyl	4
8	Redigo	prothioconazole	2.5
	Trilex FL	trifloxystrobin	2.5
	Allegiance FL	metalaxyl	4
9	Redigo	prothioconazole	5
	Trilex AL	trifloxystrobin + metalaxyl	5 + 4
	Yield Shield	<i>Bacillus pumilus</i>	17,400 cfu
10	Topsin M 4.5 FL	thiophanate-methyl	354
	Apron XL	mefenoxam	3.75
11	BAS 580	pyraclostrobin + thiophanate-methyl	5 + 45
	Apron XL	metalaxyl	3.75
12	Topsin M 4.5 FL	thiophanate-methyl	354
	Dynasty	azoxystrobin	3
	Yield Shield	<i>Bacillus pumilus</i>	17,400 cfu
	Redigo	prothioconazole	5
	Maxim 4 FS	fludioxonil	5
	Apron XL	mefenoxam	3.75

\* Active ingredient is a bacterium, and the rate is expressed in colony forming units (CFU).

Table 2. The seed treatments and rates used in the rolled-towel laboratory experiment.

Seed treatment			Grams a. i. per 100 kg Seed
#	Trade name	Active ingredient	
1		non-treated/non-inoculated <sup>a</sup>	
2		NaOCl <sup>b</sup> /non-inoculated <sup>a</sup>	
3		non-treated	
4		NaOCl <sup>b</sup>	
5	Apron XL	mefenoxam	3.75
6	Dynasty	azoxystrobin	3
7	Yield Shield	<i>Bacillus pumilus</i>	17,400 cfu*
8	Maxim 4 FS	fludioxonil	5
9	Redigo	prothioconazole	5
10	Stamina	pyraclostrobin	5
11	BAS 580	pyraclostrobin + thiophanate-methyl	5 + 45
12	Topsin M 4.5 FL	thiophanate-methyl	354
13	Trilex FL	trifloxystrobin	2.5
14	Dynasty	azoxystrobin	3
	Yield Shield	<i>Bacillus pumilus</i>	17,400 cfu*
	Maxim 4 FS	fludioxonil	5
	Apron XL	mefenoxam	3.75
	Redigo	prothioconazole	5
	Topsin M 4.5 FL	thiophanate-methyl	354

\* Active ingredient is a bacterium, and the rate is expressed in colony forming units (CFU).

<sup>a</sup> Seeds were not inoculated with 100 µl of a  $2.5 \times 10^5$  *F. virguliforme* conidial solution, while all other seeds were inoculated.

<sup>b</sup> Prior to the experiment, NaOCl (0.5%) was used to rinse seeds for 90 seconds. Then, seeds were rinsed twice with sterilized, distilled water for 90 seconds to remove NaOCl.

Table 3. Summary of main effects and interactions on soybean stand count, Fv DNA concentration in roots, SDS area under disease progress curve (AUDPC), seed moisture, 100-seed mass, and yield at Valmeyer, IL in 2008.

Source	Plant Stand	Fv DNA <sup>a</sup>	SDS <sup>b</sup>	Yield	100-seed weight	Seed moisture
Cultivar (C)	<0.0001 <sup>c</sup>	0.5674	<0.0001	<0.0001	<0.0001	0.1762
Seed Treatment (S)	0.0002	0.4670	0.0025	0.1913	0.0664	0.2992
C X S	0.2562	0.9865	0.2577	0.7787	0.0092	0.8731

<sup>a</sup> Natural log transformations of [values x 10] were used to meet assumptions of normality for analysis.

<sup>b</sup> AUDPC calculated by:  $AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = the disease index percent rating at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations.

<sup>c</sup> Numbers are the P>F values, alpha = 0.05.

Table 4. Summary of main effects and interactions on root length, root surface area, average root diameter, root volume, number of root tips, and number of root forks at the V4, V7, and V12 developmental stages analyzed with WinRHIZO 2007 Root Analysis Software at Valmeyer, IL in 2008.

Root timing	Source	Length	Surface area	Average diameter	Volume	Tips	Forks
V4	Cultivar (C)	0.0242 <sup>a</sup>	0.0009	0.2095	0.0003	0.9617	0.0151
	Seed Treatment (S)	0.5184	0.3687	0.8914	0.0555	0.9428	0.7683
	C X S	0.0489	0.0521	0.6921	0.5488	0.1756	0.1862
V7	Cultivar (C)	0.0062	0.0567	0.0393	0.3223	0.3151	0.0115
	Seed Treatment (S)	0.8607	0.5040	0.0451	0.1252	0.0052	0.8229
	C X S	0.7781	0.6790	0.0133	0.1356	0.0130	0.8979
V12	Cultivar (C)	0.2687	0.7409	0.2657	0.7991	0.5836	0.6460
	Seed Treatment (S)	0.2835	0.6906	0.3751	0.7960	0.2660	0.2791
	C X S	0.9187	0.9614	0.8026	0.9054	0.6849	0.7186

<sup>a</sup> Numbers are the P>F values, alpha = 0.05.

Table 5. Effects of seed treatments on plant stand, SDS area under disease progress curve (AUDPC), and yield at Valmeyer, IL in 2008.

Seed treatment	Plant stand (plants/m <sup>2</sup> )	SDS (AUDPC) <sup>a</sup>	Yield (kg/ha)
non-treated	26.8 d <sup>b</sup>	252 a	4,428 bc
mefenoxam	28.1 cd	201 bc	4,049 c
mefenoxam, fludioxonil	32.4 a	169 bcd	5,046 a
mefenoxam, azoxystrobin	31.0 ab	149 d	4,667 ab
mefenoxam, fludioxonil, azoxystrobin	31.3 ab	194 bcd	4,355 bc
trifloxystrobin + metalaxyl	31.1 ab	198 bcd	4,691 ab
metalaxyl, prothioconazole	30.6 ab	152 cd	4,780 ab
metalaxyl, prothioconazole, trifloxystrobin	30.6 ab	217 ab	4,364 bc
prothioconazole, trifloxystrobin + metalaxyl	29.9 bc	159 cd	4,561 abc
<i>B. pumilus</i>			
mefenoxam, thiophanate-methyl	30.9 ab	163cd	4,585 abc
metalaxyl, pyraclostrobin + thiophanate-methyl	31.5 ab	175 bcd	4,517 abc
mefenoxam, thiophanate-methyl, azoxystrobin,	27.8 cd	174 bcd	4,495 abc
<i>B. pumilus</i> , prothioconazole, fludioxonil			

<sup>a</sup> AUDPC calculated by:  $AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = the disease index percent rating at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations.

<sup>b</sup> Estimates from PROC MIXED were used to identify differences between seed treatments at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.



Table 6. Effects of cultivars and seed treatments on 100-seed weight.

Seed treatment	100-seed weight (g)	
	FS 4456 <sup>a</sup>	Pioneer 94M30 <sup>a</sup>
non-treated	13.6 a <sup>b</sup>	16.8 cde
mefenoxam	13.5 ab	17.1 cde
mefenoxam, fludioxonil	13.3 ab	17.6 abc
mefenoxam, azoxystrobin	12.8 ab	17.5 abc
mefenoxam, fludioxonil, azoxystrobin	13.1 ab	16.8 cde
trifloxystrobin + metalaxyl	13.2 ab	16.9 cde
metalaxyl, prothioconazole	13.5 ab	17.3 bcd
metalaxyl, prothioconazole, trifloxystrobin	12.7 b	17.9 ab
prothioconazole, trifloxystrobin + metalaxyl	13.0 ab	16.5 e
<i>B. pumilus</i>		
mefenoxam, thiophanate-methyl	13.3 ab	18.3 a
metalaxyl, pyraclostrobin + thiophanate-methyl	13.2 ab	16.6 de
mefenoxam, thiophanate-methyl, azoxystrobin,	12.8 b	17.1 bcde
<i>B. pumilus</i> , prothioconazole, fludioxonil		

<sup>a</sup> Cultivar FS 4456 and Pioneer 94M30 are considered moderately-susceptible (MS) and moderately-resistant (MR) to sudden death syndrome, respectively.

<sup>b</sup> Estimates from PROC MIXED were used to identify differences between seed treatments in cultivars at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.

Table 7. Effects of cultivars and seed treatments on root length at the V4 developmental stage at Valmeyer, IL in 2008.

Seed treatment	Length (cm)	
	FS 4456 <sup>a</sup>	Pioneer 94M30 <sup>a</sup>
non-treated	112.4 ab <sup>b</sup>	149.8 ab
mefenoxam	102.6 ab	132.8 abcd
mefenoxam, fludioxonil	113.5 ab	112.6 cd
mefenoxam, azoxystrobin	124.5 ab	132.4 abcd
mefenoxam, fludioxonil, azoxystrobin	98.1 b	136.5 abc
trifloxystrobin + metalaxyl	121.2 ab	133.0 abcd
metalaxyl, prothioconazole	106.3 ab	163.1 a
metalaxyl, prothioconazole, trifloxystrobin	119.8 ab	112.5 cd
prothioconazole, trifloxystrobin + metalaxyl	110.3 ab	114.4 cd
<i>B. pumilus</i>		
mefenoxam, thiophanate-methyl	108.0 ab	110.4 cd
metalaxyl, pyraclostrobin + thiophanate-methyl	134.0 a	123.5 bcd
mefenoxam, thiophanate-methyl, azoxystrobin,	130.6 ab	101.0 d
<i>B. pumilus</i> , prothioconazole, fludioxonil		

<sup>a</sup> Cultivar FS 4456 and Pioneer 94M30 are considered moderately-susceptible (MS) and moderately-resistant (MR) to sudden death syndrome, respectively.

<sup>b</sup> Estimates from PROC MIXED were used to identify differences between seed treatments in cultivars at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.

Table 8. Effects of cultivars and seed treatments on average root diameter and number of root tips at the V7 developmental stage at Valmeyer, IL in 2008.

Seed treatment	Average diameter (mm)		Tips (no.)	
	FS 4456 <sup>a</sup>	Pioneer 94M30 <sup>a</sup>	FS 4456	Pioneer 94M30
non-treated	0.90 abc <sup>b</sup>	0.98 a	848 abc	648 de
mefenoxam	0.93 ab	0.96 ab	910 ab	693 cde
mefenoxam, fludioxonil	0.95 a	0.89 abc	566 c	607 e
mefenoxam, azoxystrobin	0.89 abc	0.86 cd	700 bc	857 cde
mefenoxam, fludioxonil, azoxystrobin	0.84 c	0.89 abc	1,072 a	668 de
trifloxystrobin + metalaxyl	0.85 bc	0.88 bcd	824 abc	818 cde
metalaxyl, prothioconazole	0.87 abc	0.86 cd	850 abc	839 cde
metalaxyl, prothioconazole, trifloxystrobin	0.89 abc	0.87 cd	934 ab	994 abc
prothioconazole, trifloxystrobin + metalaxyl	0.94 a	0.80 d	874 abc	1,286 a
<i>B. pumilus</i>				
mefenoxam, thiophanate-methyl	0.92 abc	0.85 cd	822 abc	1,213 ab
metalaxyl, pyraclostrobin + thiophanate-methyl	0.92 abc	0.84 cd	674 bc	941 bcd
mefenoxam, thiophanate-methyl, azoxystrobin,	0.92 ab	0.84 cd	780 abc	841 cde
<i>B. pumilus</i> , prothioconazole, fludioxonil				

<sup>a</sup> Cultivar FS 4456 and Pioneer 94M30 are considered moderately-susceptible (MS) and moderately-resistant (MR) to sudden death syndrome, respectively.

<sup>b</sup> Estimates from PROC MIXED were used to identify differences between seed treatments in cultivars at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.

Table 9. Summary of main effects and interactions on soybean plant stand, Fv DNA concentration in roots, 2009 SDS area under disease progress curve (AUDPC), yield, 100-seed mass, and seed moisture at Urbana, IL in 2008 and 2009.

Source	Plant stand	Fv DNA <sup>a</sup>	SDS <sup>bc</sup>	Yield	100-seed weight	Seed moisture <sup>d</sup>
Year (Y)	0.1778 <sup>e</sup>	0.4725		-	0.1082	0.3078
Cultivar (C)	-	0.7100	<0.0001	0.4778	0.4639	0.7860
Y X C	0.0097	0.4559		0.0058	0.0488	<0.0001
Seed Treatment (S)	0.3594	0.6142	0.1224	0.2375	0.2327	0.5064
Y X S	0.1770	0.4640		-	-	-
C X S	0.9659	0.3876	0.4528	0.8252	0.6770	0.2424
Y X C X S	0.8597	0.9552		-	0.8967	0.8236

- Covariance parameter estimate was equal to zero. Term is not significant.

<sup>a</sup> Natural log transformations of [values + 1] were used to meet assumption of normality for analysis.

<sup>b</sup> Sudden death syndrome foliar symptoms were only present in 2009 for disease rating and analysis.

<sup>c</sup> AUDPC calculated by:  $AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = the disease index percent rating at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations.

<sup>d</sup> Sine transformations of seed moisture values were used to meet assumptions of normality for analysis.

<sup>e</sup> Numbers are the P>F values, alpha = 0.05.

Table 10. Summary of main effects and interactions on root length, root surface area, average root diameter, root volume, number of root tips, and number of root forks at the V1, V3, and V6 developmental stages analyzed with WinRHIZO 2007 Root Analysis Software at Urbana, IL in 2008 and 2009.

Root timing	Source	Length	Surface area	Average diameter <sup>a</sup>	Volume <sup>b</sup>	Tips <sup>c</sup>	Forks <sup>d</sup>
V1	Year (Y)	0.5723 <sup>e</sup>	0.1067	-	0.0523	0.0015	-
	Cultivar (C )	0.0009	<0.0001	0.0745	0.1550	0.3053	0.1113
	Y X C	-	-	-	-	<0.0001	0.9934
	Seed Treatment (S)	0.4774	0.5230	0.1730	0.4008	0.5760	0.4754
	Y X S	0.5478	0.0953	-	-	-	0.9633
	C X S	0.5564	0.2583	0.1986	0.0405	0.8184	0.7044
	Y X C X S	0.9422	-	0.9677	-	0.9608	0.9431
V3	Year (Y)	0.0250	<0.0001	-	0.0531	0.0004	0.0025
	Cultivar (C )	0.2917	0.0004	0.2524	0.6845	0.2152	0.2076
	Y X C	0.0001	-	0.8606	<0.0001	<0.0001	0.0005
	Seed Treatment (S)	0.8354	0.9409	0.3176	0.7288	0.1562	0.9595
	Y X S	-	-	-	-	-	0.0026
	C X S	0.9320	0.7790	0.4949	0.2359	0.6544	0.9675
	Y X C X S	0.9214	0.9936	0.8572	-	-	0.9509
V6	Year (Y)	0.0771	0.0704	0.0033	0.0939	<0.0001	0.0515
	Cultivar (C )	0.2725	0.3800	0.1140	0.9256	0.1534	0.2501
	Y X C	<0.0001	<0.0001	0.0017	0.0092	-	<0.0001
	Seed Treatment (S)	0.5059	0.7572	0.1734	0.7271	0.5344	0.7155
	Y X S	-	-	-	-	-	-
	C X S	0.9473	0.9822	0.1060	0.8813	0.4382	0.9450
	Y X C X S	0.9143	0.9160	-	0.9207	-	0.9351

Table 10. (Continued)

- Covariance parameter estimate is equal to zero. The term is not significant.

<sup>a</sup> Natural log transformations of [average root diameter values x 10] were used to meet assumptions of normality for analysis at developmental stages V1 and V3. Average root diameter values were not transformed at developmental stage V6.

<sup>b</sup> Root volumes were not transformed at developmental stage V1. Square root transformations of root volume values were used to meet assumptions of normality for analysis at developmental stages V3 and V6.

<sup>c</sup> Natural log transformations of number of root tip values were used to meet assumptions of normality for analysis at developmental stages V1 and V3. Square root transformations of number of root tip values were used to meet assumptions of normality for analysis at developmental stage V6.

<sup>d</sup> Square root transformations of number of root fork values were used to meet assumptions of normality for analysis at developmental stage V1, V3 and V6.

<sup>e</sup> Numbers are the P>F values, alpha = 0.05.

Table 11. Effects of years and cultivars on plant stand, yield, 100-seed weight, and seed moisture at Urbana, IL in 2008 and 2009.

Year	Cultivar <sup>a</sup>	Plant stand (plants/m <sup>2</sup> )	Yield (kg/ha)	100-seed weight (g)	Seed moisture <sup>b</sup> (%)
2008	FS 3466	33.7 a <sup>c</sup>	3,417 b	15.7 a	12.9 a
	NK 33-A8	35.3 a	3,555 a	15.8 a	11.5 b
2009	FS 3466	40.8 a	3,650 a	16.6 b	13.2 b
	NK 33-A8	39.2 a	2,899 b	17.1 a	13.7 a

<sup>a</sup> Cultivar FS 3466 and NK 33-A8 are considered moderately-resistant (MR) and moderately-susceptible (MS) to sudden death syndrome, respectively.

<sup>b</sup> Sine transformations of values were used to meet assumption of normality for analysis. Non-transformed means are displayed.

<sup>c</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between cultivars in trials at alpha = 0.05, values followed by the same letter within a year and column are not significantly different from each other.

Table 12. Effect of soybean seed treatments and cultivars on root volume at the V1 developmental stage at Urbana, IL averaged across 2008 and 2009.

Seed treatment	Volume (cm <sup>3</sup> )	
	FS 3466 <sup>a</sup>	NK 33-A8 <sup>a</sup>
non-treated	0.37 ab <sup>b</sup>	0.30 c
mefenoxam	0.36 b	0.39 a
mefenoxam, fludioxonil	0.39 ab	0.34 abc
mefenoxam, azoxystrobin	0.39 ab	0.36 ab
mefenoxam, fludioxonil, azoxystrobin	0.41 ab	0.34 abc
trifloxystrobin + metalaxyl	0.38 ab	0.33 bc
metalaxyl, prothioconazole	0.43 a	0.32 bc
metalaxyl, prothioconazole, trifloxystrobin	0.38 ab	0.37 ab
prothioconazole, trifloxystrobin + metalaxyl,	0.37 ab	0.31 bc
<i>B. pumilus</i>		
mefenoxam, thiophanate-methyl	0.36 b	0.39 a
metalaxyl, pyraclostrobin + thiophanate-methyl	0.40 ab	0.37 ab
mefenoxam, thiophanate-methyl, azoxystrobin,	0.37 b	0.34 abc
<i>B. pumilus</i> , prothioconazole, fludioxonil		

<sup>a</sup> Cultivar FS 3466 and NK 33-A8 are considered moderately-resistant (MR) and moderately-susceptible (MS) to sudden death syndrome, respectively.

<sup>b</sup> Estimates from the mixed models procedure were used to identify differences among seed treatments within cultivars at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.



Table 13. Effects of years and cultivars on root length, root volume, number of root tips, and number of root forks at the V3 developmental stage at Urbana, IL in 2008 and 2009.

Year	Cultivar <sup>a</sup>	Length (cm)	Volume <sup>b</sup> (cm <sup>3</sup> )	Tips <sup>c</sup> (no.)	Forks <sup>b</sup> (no.)
2008	FS 3466	104.4 a <sup>d</sup>	0.68 a	380 a	349 a
	NK 33-A8	84.8 b	0.70 a	288 b	277 b
2009	FS 3466	51.0 a	0.36 a	113 a	156 a
	NK 33-A8	43.0 a	0.31 b	96 b	123 a

<sup>a</sup> Cultivar FS 3466 and NK 33-A8 are considered moderately-resistant (MR) and moderately-susceptible (MS) to sudden death syndrome, respectively.

<sup>b</sup> Square root transformations of values were used to meet assumption of normality for analysis. Non-transformed means are displayed.

<sup>c</sup> Natural log transformations of values were used to meet assumption of normality for analysis. Non-transformed means are displayed.

<sup>d</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between cultivars in trials at alpha = 0.05, values followed by the same letter within a year and column are not significantly different from each other.

Table 14. Effects of years and seed treatments on number of root forks at the V3 developmental stage at Urbana, IL in 2008 and 2009.

Seed treatment	Forks <sup>a</sup> (no.)	
	2008	2009
non-treated	308 a <sup>b</sup>	135 a
mefenoxam	291 a	125 a
mefenoxam, fludioxonil	306 a	137 a
mefenoxam, azoxystrobin	313 a	141 a
mefenoxam, fludioxonil, azoxystrobin	342 a	147 a
trifloxystrobin + metalaxyl	314 a	144 a
metalaxyl, prothioconazole	302 a	135 a
metalaxyl, prothioconazole, trifloxystrobin	342 a	165 a
prothioconazole, trifloxystrobin + metalaxyl,	304 a	136 a
<i>B. pumilus</i>		
mefenoxam, thiophanate-methyl	301 a	133 a
metalaxyl, pyraclostrobin + thiophanate-methyl	293 a	137 a
mefenoxam, thiophanate-methyl, azoxystrobin,	324 a	140 a
<i>B. pumilus</i> , prothioconazole, fludioxonil		

<sup>a</sup> Square root transformations of values were used to meet assumption of normality for analysis. Non-transformed means are displayed.

<sup>b</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between seed treatments in trials at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.

Table 15. Effects of years and cultivars on root length, root surface area, average root diameter, root volume and number of root forks at the V6 developmental stage at Urbana, IL in 2008 and 2009.

Year	Cultivar <sup>a</sup>	Length (cm)	Surface area (cm <sup>2</sup> )	Average diameter (mm)	Volume <sup>b</sup> (cm <sup>3</sup> )	Forks <sup>b</sup> (no.)
2008	FS 3466	241.6 a <sup>c</sup>	66.42 a	0.89 a	1.45 a	1,047 a
	NK 33-A8	198.1 b	59.81 b	0.97 a	1.45 a	753 b
2009	FS 3466	137.3 a	45.45 a	1.07 b	1.22 a	308 a
	NK 33-A8	118.0 b	43.07 a	1.16 a	1.23 a	236 b

<sup>a</sup> Cultivars FS 3466 and NK 33-A8 are considered moderately-resistant (MR) and moderately-susceptible (MS) to sudden death syndrome, respectively.

<sup>b</sup> Square root transformations of values were used to meet assumption of normality for analysis. Non-transformed means are displayed.

<sup>c</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between cultivars in trials at alpha = 0.05, values followed by the same letter within a year and column are not significantly different from each other.

Table 16. Summary of main effects and interactions on plant stand, SDS area under disease progress curve (AUDPC), Fv DNA concentration in roots, root length, root surface area, average root diameter, root volume, number of root tips, and number of root forks analyzed with WinRHIZO 2007 Root Analysis software, from greenhouse based studies.

Source	Plant stand	SDS <sup>a</sup>	Fv DNA <sup>b</sup>	Length <sup>b</sup>	Root analysis				
					Surface area <sup>b</sup>	Average diameter	Volume <sup>c</sup>	Tips <sup>b</sup>	Forks <sup>b</sup>
Trial (T)	0.7877 <sup>d</sup>	0.1385	0.0079	0.0300	0.2859	0.0166	-	<0.0001	0.0289
Cultivar (C )	0.6675	0.7141	0.4688	0.1354	0.2628	0.5698	0.2457	0.0069	0.1020
T X C	<0.0001	<0.0001	<0.0001	<0.0001	0.0010	<0.0001	0.9342	-	<0.0001
Seed Treatment (S)	0.4329	0.7032	0.1296	0.9652	0.9796	0.8529	0.9269	0.8482	0.8966
T X S	0.7859	0.1401	0.0111	0.0257	-	0.0197	-	-	0.0234
C X S	0.6913	0.7062	0.7804	0.1064	0.1284	0.6016	0.1296	0.5512	0.1440
T X C X S	-	-	0.9355	-	-	-	-	0.9893	-

- Covariance parameter estimate equal to zero. The term is not significant.

<sup>a</sup> AUDPC calculated by:  $AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = the disease index percent rating at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations.

<sup>b</sup> Square root transformations of values were used to meet assumption of normality for analysis.

<sup>c</sup> Natural log transformations of [values x 10] were used to meet assumption of normality for analysis.

<sup>d</sup> Numbers are the P>F values, alpha = 0.05.

Table 17. Effects of trials and cultivars on stand count, area under disease progress curve (AUDPC), and Fv DNA concentration in roots, root length, root surface area, average root diameter, and number of root forks from greenhouse based studies.

Trial	Cultivar <sup>a</sup>	Plant stand <sup>b</sup> (no./plot)	SDS <sup>c</sup> (AUDPC)	Fv DNA (pg /mg root)	Root Analysis			
					Length <sup>d</sup> (cm)	Surface area <sup>d</sup> (cm <sup>2</sup> )	Average diameter <sup>e</sup> (mm)	Forks <sup>d</sup> (no.)
Trial 1	FS 3466	8.0 b <sup>f</sup>	358 b	19,237 b	231.6 a	25.9 a	0.118 b	615 a
	NK 33-A8	9.4 a	420 a	29,789 a	169.6 a	18.7 b	0.119 ab	419 a
	Pioneer 94M30	6.5 c	360 b	16,752 b	249.3 a	29.4 a	0.128 a	745 a
Trial 2	FS 3466	9.2 a	483 b	74,622 a	159.2 a	21.7 a	0.153 b	376 a
	NK 33-A8	9.2 a	492 b	73,046 a	119.9 a	17.8 a	0.169 a	253 a
	Pioneer 94M30	9.6 a	584 a	72,458 a	147.0 a	20.2 a	0.159 ab	375 a

<sup>a</sup> Cultivars FS 3466, Pioneer 94M30 are considered moderately-resistant (MR) to sudden death syndrome. NK 33-A9 is considered moderately-susceptible (MS) to sudden death syndrome.

<sup>b</sup> Each plot was planted with ten seed. Emerged seedling were counted at approximately 14 DAP.

<sup>c</sup> AUDPC calculated by:  $AUDPC = \sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2][t_{i+1} - t_i]$ , in which  $X_i$  = the disease index percent rating at the  $i$ th observation,  $t_i$  = time (days between ratings), and  $n$  = total number of observations.

<sup>d</sup> Square Root transformations of values were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>e</sup> Log transformations of values were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>f</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between treatments in trials at alpha = 0.05, values followed by the same letter within a trial and column are not significantly different from each other.

Table 18. Effect of trials and seed treatments on Fv concentration in roots, root length, average root diameter, root volume, number of root tips, and number of root forks from greenhouse based studies.

Seed Treatment	Fv DNA <sup>a</sup>		Root analysis					
	(pg/mg root)		Length <sup>a</sup>		Average diameter <sup>b</sup>		Fork <sup>a</sup>	
	Trial 1	Trial 2	(cm)		(mm)		(no.)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
non-treated	26,043 a <sup>c</sup>	75,334 ab	225.7 a	132.6 a	0.12 a	0.16 a	635 a	327 a
mefenoxam	27,096 a	84,181 a	250.6 a	135.4 a	0.13 a	0.16 a	770 a	291 a
mefenoxam, fludioxonil	20,466 ab	74,545 ab	208.1 a	151.3 a	0.12 a	0.17 a	607 a	347 a
mefenoxam, azoxystrobin	23,519 a	76,358 ab	186.1 a	118.9 a	0.13 a	0.17 a	437 a	278 a
mefenoxam, fludioxonil, azoxystrobin	22,350 a	73,289 ab	211.6 a	143.5 a	0.13 a	0.16 a	554 a	354 a
trifloxystrobin + metalaxyl	19,566 ab	68,377 ab	236.9 a	117.8 a	0.12 a	0.16 a	649 a	246 a
metalaxyl, prothioconazole	18,085 ab	67,897 ab	204.1 a	135.9 a	0.12 a	0.16 a	510 a	311 a
metalaxyl, prothioconazole, trifloxystrobin	21,095 a	75,043 ab	240.5 a	112.7 a	0.12 a	0.17 a	675 a	262 a
prothioconazole, trifloxystrobin + metalaxyl,	22,714 a	79,772 a	239.3 a	133.4 a	0.12 a	0.15 a	673 a	309 a
<i>B. pumilus</i>								
mefenoxam, thiophanate-methyl	16,905 ab	58,105 bc	227.0 a	176.2 a	0.13 a	0.15 a	644 a	470 a
metalaxyl, pyraclostrobin + thiophanate-methyl	22,076 a	71,412 ab	208.0 a	140.0 a	0.12 a	0.17 a	541 a	330 a
mefenoxam, thiophanate-methyl, azoxystrobin,	11,259 b	48,633 c	164.3 a	206.5 a	0.13 a	0.15 a	416 a	495 a
<i>B. pumilus</i> , prothioconazole, fludioxonil								

<sup>a</sup> Square Root transformations of values were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>b</sup> Log transformations of [values + 1] were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>c</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between seed treatments in trials at alpha = 0.05, values followed by the same letter within a column are not significantly different from each other.

Table 19. Summary of seed treatment main effects and interactions on seed germination, seedling length, lesion length, and disease severity in the rolled-towel laboratory assay.

Source	Seed germination <sup>a</sup>	Plant length	Lesion length <sup>b</sup>	Disease severity <sup>b</sup>
Trial (T)	- <sup>c</sup>	0.0212	0.1130	0.1006
Seed Treatment (S)	0.0453	0.0041	0.0328	0.0035
T X S	0.5693	0.0006	<0.0001	0.0004

- Covariance parameter estimate is equal to zero. The term is not significant.

<sup>a</sup> Square root transformations of values were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>b</sup> Natural log transformations of [values + 1] were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>c</sup> Numbers are the P>F values, alpha = 0.05.

Table 20. Effect of seed treatments on seed germination in the rolled-towel laboratory assay.

Seed treatment	Seed germination <sup>a</sup> (%)
non-treated/non-inoculated	95 ab <sup>b</sup>
NaOCl/non-inoculated	93 abc
non-treated	89 abc
NaOCl	94 abc
mefenoxam	87 abc
azoxystrobin	88 abc
<i>B. pumilus</i>	57 d
fludioxonil	97 a
prothioconazole	93 abc
pyraclostrobin	78 cd
pyraclostrobin + thiophanate-methyl	91 abc
thiophanate-methyl	79 bcd
trifloxystrobin	81 bcd
azoxystrobin, <i>B. pumilus</i> , fludioxonil, mefenoxam, prothioconazole, thiophanate-methyl	82 bcd

<sup>a</sup> Square root transformations of values were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>b</sup> Estimates from PROC MIXED were used to identify differences between seed treatments at alpha = 0.05, values followed by the same letter are not significantly different from each other.



Table 21. Effect of trials and seed treatments on plant length, lesion length, and disease severity in the rolled-towel laboratory assay.

Seed treatment	Plant length (cm)		Lesion length (cm) <sup>a</sup>		Disease severity (%) <sup>a</sup>	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
non-treated/non-inoculated <sup>b</sup>	25.2 ab <sup>d</sup>	27.7 abcde	3.65 bcd	0.73 def	25.7 cd	5.9 de
NaOCl/non-inoculated <sup>bc</sup>	28.3 a	33.0 a	0.60 e	1.38 bcde	4.9 f	4.0 ef
non-treated	21.5 abcd	25.3 bcdef	2.40 d	2.71 a	29.0 bcd	28.7 abc
NaOCl <sup>c</sup>	21.5 abcd	29.6 abc	6.55 a	2.23 a	44.2 abc	13.6 bcd
mefenoxam	17.8 cd	22.1 defg	2.83 cd	2.56 a	36.3 abc	29.4 ab
azoxystrobin	23.5 abc	28.1 abcd	5.39 ab	1.44 bcd	40.4 abc	9.6 cd
<i>B. pumilus</i>	8.1 e	12.3 h	3.16 bcd	1.89 abc	69.6 a	41.4 a
fludioxonil	25.4 ab	29.7 abc	0.84 e	0.58 ef	8.7 ef	9.1 de
prothioconazole	16.0 d	17.5 gh	4.71 abc	2.02 ab	31.3 abc	34.4 ab
pyraclostrobin	16.3 d	22.8 cdefg	4.81 abc	0.96 cde	55.6 ab	18.8 abc
pyraclostrobin + thiophanate-methyl	20.3 bcd	23.2 cdefg	0.55 e	0.30 f	20.9 cd	10.9 cd
thiophanate-methyl	19.6 bcd	20.9 efg	0.36 e	1.03 de	14.2 de	29.4 abc
trifloxystrobin	14.9 de	18.3 fgh	2.78 cd	2.77 a	33.6 abc	38.3 ab
azoxystrobin, <i>B. pumilus</i> , fludioxonil, mefenoxam, prothioconazole, thiophanate-methyl	24.5 abc	30.8 ab	0.83 e	0.40 f	10.1 ef	1.3 f

<sup>a</sup> Natural log transformations of [values + 1] were used to meet assumptions of normality for analysis. Non-transformed means are displayed.

<sup>b</sup> Seeds were not inoculated with 100 µl of 2.5 x 10<sup>5</sup> Fv conidial solution, while all other seed treatments were inoculated.

<sup>c</sup> Before the experiment, NaOCl (0.5%) was used to rinse seeds for 90 seconds. Then, seeds were rinsed twice with distilled water to remove NaOCl.

<sup>d</sup> Best Linear Unbiased Predictions (BLUPs) from PROC MIXED were used to identify differences between seed treatments in trials at alpha = 0.05, values followed by the same letter within a year and column are not significantly different from each other.

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